Inclusion Complex of Thiourea Substrate with Hydroxypropyl- β -cyclodextrin

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Abstract: To be active, a heavy metal trap substrate must possess some degree of aqueous solubility. The use of cyclodextrins molecules, which can form inclusion complexes with the hydrophobic trap substrates, has provided the required solubility through the relatively hydrophobic cavity of native cyclodextrins and their derivatives that enhances the ability to complex hydrophobic substrates of appropriate size and shape. In this study, spectrophotometry has been used to investigate the effects of cyclodextrin on the solubility, stoichiometry and stability of the substrate-CD complex formed between *N*-(2-ethylphenyl)-*N'*-(2-methylphenyl) thiourea, (a hydrophobic substrate trap) and hydroxypropyl- β -cyclodextrin (HP- β -CD). The solubility, stoichiometry and overall association constant of the complex have been determined using the Higuchi's method, the continuous variation (Job's plot) method and the Scott's method respectively.

Keywords: Thiourea, Inclusion complexes, Cyclodextrin

Introduction

The literature is rich with research investigating the effects of heavy metals on the human health¹⁻⁴. *N*-(2-Ethylphenyl)-*N'*-(2-methylphenyl) thiourea belongs to the thiourea compounds family which are active traps of the heavy metals and play an important role in the field of asymmetric organocatalysis⁵. Bifunctional chiral thioureas have emerged as a successful class of organocatalysts for a variety of asymmetric transformations, such as the cyanosilylation of ketones⁶, Strecker reactions⁷, Michael additions⁸ and hydrophosphonylation of imines⁹. Their success as bifunctional organocatalysts is based upon their ability to function as hydrogen-bond donors and the incorporation of additional basic or acidic activating groups¹⁰.

Cyclodextrins (CD) are a family of cyclic oligosaccharides containing 6(α), 7(β), 8(γ) or more glucose residues and are characterized by a truncated cone shape¹¹. Cyclodextrin molecules consist of secondary hydroxyl groups extending from the wider edge while the

primary groups from the narrow edge. This gives cyclodextrin molecules a hydrophilic outer surface, whereas the lipophilicity of their central cavity is comparable to an aqueous ethanolic solution^{12,13}. Natural cyclodextrins show limited aqueous solubility due to the strong intermolecular hydrogen bonding in the crystal state. Substitution of any of the hydrogen bond-forming hydroxyl groups, greatly improves their aqueous solubility¹⁴. CD derivatives of interest include the hydroxypropyl derivatives (*i.e.* HP- α -CD, HP- β -CD and HP- γ -CD), the randomly methylated-CD and sulfobutylether-CD¹⁵⁻¹⁹.

Cyclodextrins are widely used as trap molecules in the pharmaceutical, agrochemical, food and cosmetic industries²⁰. They (example: Figure 1a) are used as complexing agents that encapsulate a variety of poorly water-soluble substrates (example: Figure 1b); completely, or some lipophilic moiety of the substrate molecule, inside their cavity through non-covalent bonds to form inclusion complexes resulting in an increase in their apparent water solubility²¹. No covalent bonds are being formed or broken during complex formation, and the substrate molecules in complex are in rapid equilibrium with free molecules in the solution. The forces involved in the complex formation are the hydrogen bonding, the Vander Waals' interaction and the charge transfer interaction²². The physicochemical properties of the free cyclodextrin molecule differ from those in the complex²³.

Figure 1. (a) Chemical structure of hydroxypropyl- β -cyclodextrin (HP- β -CD); (b) *N*-(2-ethylphenyl)-*N'*-(2-methylphenyl) thiourea substrate (the heavy metal trap)

Despite several successful methods, including NMR spectroscopy²⁴, mass spectrometry and molecular modelling²⁵, the spectrophotometric method (employed in this study) still remains the simplest and most rapid technique for the determination of the solubility, the stoichiometry and the association constant for *N*-(2-ethylphenyl)-*N'*-(2-methylphenyl) thiourea substrate with HP- β -CD.

Experimental

The preparation and purification of *N*-(2-ethylphenyl)-*N'*-(2-methylphenyl) thiourea substrate has been prepared according to the procedure reported in the literature²⁶. HP- β -CD and hexane of analytical grade have been purchased from Sigma-Aldrich Company. All solvents and materials used throughout this study are of analytical grade and used as such. All laboratory reagents have been freshly prepared. Water has been purified by triple distillation.

Apparatus

Double beam UV-1800 (Schimadzu UV-Vis, Japan) spectrophotometer with matched 1 cm quartz cells has been used for all the spectrophotometric measurements. The wavelength of UV detector has been set at 190 nm.

Preparation of solutions

Substrate has been accurately weighted, transferred to volumetric flasks and dissolved in water to make individual stock solutions of 10 mmol/L. The stock solutions have been stored at 4 °C and have been further diluted with water to obtain working solutions.

Preparation of inclusion complexes

For each measurement, cyclodextrin and substrate have been mixed and shaken at the temperature of 25 °C to obtain a stable state of solubilization.

Partition coefficient determination

The lipophilicity of the substrate has been evaluated from their *n*-hexane-water partition coefficient, K_p , as follows: Equal volumes of freshly prepared substrate/hexane solution (10 mmol/L) and of triple distilled water have been mixed and vigorously stirred at 25 °C for 1 h. The two phases have been separated by brief centrifugation (1000 g for 20 s). The substrate concentration in either the hexanic or the aqueous phase has been determined by a Schimadzu UV-VIS spectrophotometer (UV-1800). K_p has been evaluated as the ratio of the substrate concentration in *n*-hexane to that in water.

Absorption spectra

Schimadzu UV-Vis spectrophotometer (UV-1800) has been employed to determine the wavelength of maximum absorption (190 nm) for the substrate in water.

Standard curves

For calibration, a 10 mmol/L mother solution of substrate in water has been used. A series of 5 mL solutions of concentrations between 0.1 and 10 mmol/L have been prepared and left at room temperature for 10 min. Absorbance has been measured at 190 nm for each solution against a blank solution. The molar extinction coefficient has been determined by measuring the absorbance as a function of concentration.

Solubility

Higuchi's method has been employed²⁷. Eight solutions each containing 10 mL of water and 100 mg of *N*-(2-ethylphenyl)-*N'*-(2-methylphenyl) thiourea substrate have been prepared. To each, different HP- β -CD concentration solutions (0, 2, 4, 6, 8, 10, 12 and 14 mmol/L) have been added. The complex formed for each reaction mixture has been allowed to stand for 24 h before filtration on a 0.2 μ m porous disc and the dissolved quantity of the complex substrate-HP- β -CD in water has been determined at 190 nm. The method is based on the graphical representation of the curves, obtained by means of the experimental measurements from a chemical system in equilibrium using origin 6.0 professional program.

Stoichiometry of the complex

Job's method of continuous variation has been employed²⁸. Starting from the initial (10 mmol/L) solutions of each HP- β -CD and the substrate, series of 5 mL quantities of HP- β -CD and the substrate have been prepared comprising different complementary proportions

(0 : 5, 0.5 : 4.5, 4.5 : 0.5 and 5 : 0). The complex formed for each reaction mixture has been allowed to stand for 10 min before analysis at 190 nm. The same procedure, described in section 2.8, has followed.

Association constant K_a

Scott's plot method has been employed²⁹. From the same initial equimolar (10 mmol/L) aqueous solutions of HP- β -CD and the substrate, serial volumes of 0 to 4.5 mL of HP- β -CD solution has been transferred to different test tubes. 0.25 mL of the substrate has been added to each test tube and completed to 5 mL by addition of the necessary volumes of water.

Results and Discussion

Solubility

In this study, the hydrophobic substrate *N*-(2-ethylphenyl)-*N'*-(2-methylphenyl) thiourea has been solubilised in water consisting of different concentrations of HP- β -CD. The effect of HP- β -CD concentration on the solubility is plotted in Figure 2. As shown, the solubility of the substrate increases with the concentration of the HP- β -CD to reach a maximum after which it remains approximately unchanged showing an equilibrium state established between the substrate, HP- β -CD and the complex.

Figure 2. Solubility of *N*-(2-ethylphenyl)-*N'*-(2-methylphenyl) thiourea with HP- β -CD inclusion complex as a function of HP- β -CD concentration

Stoichiometry of the complex

The stoichiometry of the complex formation between substrate and HP- β -CD has been investigated using Job's method of the continuous variation²⁸. Keeping the sum of the molar concentrations of substrate and HP- β -CD fixed, the ratio of the concentrations of the two substances in the mixture has been varied and the absorbance of the mixtures has been recorded at 190 nm against a convenient blank solution prepared for each point of the experiment. As shown in Figure 3, the maximum absorbance has been observed at a substrate

molar ratio of 0.5, indicating that it reacts with HP- β -CD in a proportion of 1:1. This is an acceptable result taking into account the size of the substrate and the presence of only one cavity in the HP- β -CD molecule.

Figure 3. Job's plot (continuous variation method) of the substrate with HP- β -CD inclusion complex showing 1:1 stoichiometry. Initial concentrations of substrate and HP- β -CD are 10 mmol/L each. Absorbance measurements were carried out at 190 nm

Determination of association constant (K_a)

Using Scott's method, the association constant (K_a) of the substrate-HP- β -CD complex has been investigated²⁹. According to Scott's equation (Eq. 1), the $[\text{HP-}\beta\text{-CD}]/\Delta A_{\text{obs}}$ has been plotted against $[\text{HP-}\beta\text{-CD}]$ (Figure 4) showing a linear correlation an indication of 1:1 inclusion complex. The slope of the plot ($1/\Delta A_{\text{max}}$) and the intercept with the vertical axis ($1/K_a \cdot \Delta A_{\text{max}}$) allow the estimation of the association constant (K_a) to be 140 M^{-1} .

$$[\text{HP-}\beta\text{-CD}]/\Delta A_{\text{obs}} = [\text{HP-}\beta\text{-CD}]/\Delta A_{\text{max}} + 1/K_a \cdot \Delta A_{\text{max}} \quad (1)$$

Figure 4. Scott's plot for substrate with HP- β -CD inclusion complex, showing overall association constant ($K_a = 140 \text{ M}^{-1}$), correlation ($R = 0.99963$), standard deviation ($\text{SD} = 2.96742 \cdot 10^{-6}$)

Partition coefficient determination

The inclusion of a substrate in cyclodextrin molecule greatly depends on its polarity. In fact, the hydrophobic characteristic will enhance the inclusion of the substrate in the hydrophobic cyclodextrin cavity^{30,31}.

The partition coefficient K_p is the ratio of the concentrations of the substrate in an organic solvent (hexane) versus that in water. Since the capacity of inclusion of the substrate in the cavity of the cyclodextrin is directly related to lipophilicity, the determination of this coefficient is essential to determine if the inclusion process is possible. Using spectrophotometric method discussed in the experimental part³², K_p has been found to be 2, which indicates a preference for *N*-(2-ethylphenyl)-*N'*-(2-methylphenyl) thiourea towards the lipid phase thus proving the possibility of penetrating the cyclodextrin cavity.

Reproducibility

The reproducibility of the methods has been determined by replicate analysis of three separate solutions of the working standard. The results have proven satisfactory with relative standard deviations not exceeding 2%.

Conclusion

Thiourea substrates are known for being traps for heavy metals and accordingly may be used as filters or as means to enhance the measurement of heavy metal concentration. The poor solubility of such substrates continues to impact the development such potential non-water soluble trap molecules candidates³³.

In this study, we have proven the possibility of increasing the solubility of the *N*-(2-ethylphenyl)-*N'*-(2-methylphenyl) thiourea by CD addition. As well, the stoichiometry and the association constant of complex have been determined. In addition, this study demonstrated that UV-Vis Spectrophotometry remains an effective, fast and accurate method for measurements aside its simplicity as compared to other methods.

References

1. Doganoc D Z, *Food Addit Contam.*, 1996, **13**(2), 237-241.
2. Lutz E, Lind B, Herin P, Krakau I, Bui T-H and Vahter M, *J Trace Elem Med Biol.*, 1996, **10**, 61-67.
3. Gerhardsson L, Brune D, Nordberg G F and Wester P O, *Sci Total Environ.*, 1986, **50**, 65-85.
4. Casey C and Robinson M F, *Br J Nutr.*, 1978, **39**(3), 639-646.
5. Dalko P I and Moisan L, *Angew Chem Int.*, 2004, **43**, 5138-5175.
6. Fuerst D E and Jacobsen E N, *J Am Chem Soc.*, 2005, **127**, 8964-8965.
7. Tsogoeva S B, Hatley M J, Yalalov D A, Meindl K, Weckbecker C and Huthmacher K, *Bioorg Med Chem.*, 2005, **13**, 5680-5685.
8. Hoashi Y, Yabuta T, Yuan P, Miyabe H and Takemoto Y, *Tetrahedron*, 2006, **62**(2-3), 365-374.
9. Joly G D and Jacobsen E N, *J Am Chem Soc.*, 2004, **126**, 4102-4103.
10. Steele M, Monti C, Gennari C, Piarulli U, Andreoli F, Vanthuyne N and Roussel C, *Tetrahedron: Asymmetry*, 2006, **17**, 999-1006.
11. Szejtli J, *Chem Rev.*, 1998, **98**, 1743-1753.
12. Szejtli J, *Cyclodextrin Technology*, Kluwer Academic Publisher, Dordrecht, 1988, Chap. 2.

13. Stella V J and Rajewski R A, *Pharm Res.*, 1997, **14**, 556-567.
14. Hakkarainen B, Fujita K, Immel S, Kenne L and Sandstrom C, *Carbohydr Res.*, 2005, **340**, 1539-1545.
15. Loftsson T and Brewster M E, *J Pharm Sci.*, 1996, **85**, 1017-1025.
16. Uekama K, Hirayama F and Irie T, *Chem Rev.*, 1998, **98**, 2045-2076.
17. Szejtli J, *Cyclodextrins and Their Inclusion Complexes*, Akademiai Kiadó, Budapest, 1982.
18. Rajewski R A and Stella V J, *J Pharm Sci.*, 1996, **85**, 1142-1169.
19. Irie T and Uekama K, *J Pharm Sci.*, 1997, **86**(11), 147-162.
20. Roux M, Perly B and Djedaini P F, *Eur Biophys J.*, 2007, **36**(8), 861-867.
21. Loftsson T and Duchêne D, *Int J Pharm.*, 2007, **329**(1-2), 1-11.
22. Liu L and Guo Q-X, *J Inclusion Phenom Macro Chem.*, 2002, **42**, 1-14.
23. Arun R, Ashok Kumar C K and Sravanthi V, *Sci Pharm.*, 2008, **76**, 567-598.
24. Mashhood Ali S and Upadhyay S K, *J Inclusion Phenom Macro Chem.*, 2008, **62**, 161-165.
25. Upadhyay S K and Kumar G, *Chem Cent J.*, 2009, **3**, 1-9, DOI:10.1186/1752-153X-3-9.
26. Bellec N, Lorcy D and Robert A, *Synthesis*, 1998, **10**, 1442-1446, doi: 10.1055/s-1998-2183.
27. Higuchi T and Connors K A, *Advances in Analytical Chemistry and instrumentation*, Reilly C N, Ed., vol. 4, Wiley-Interscience: New York, 1965.
28. Job P, *Advanced Physicochemical Experiments*, Oliner and Boyd, Edinburgh, UK, 2nd Edition, 1964.
29. Scott R L, *Recueil des Travaux Chimiques des Pays-Bas*, 1956, **75**, 787, DOI: 10.1002/recl.19560750711
30. Butkus E, Martins J C and Berg U, *J Inclusion Phenom Macrocyclic Chem.*, 1996, **26**, 209-218.
31. Grillo R, de Melo N F, Moraes C M, de Lima R, Menezes C M and Ferreira E I, *J Pharm Biomed Anal.*, 2008, **47**(2), 295-302.
32. Petracek F J and Zekhmeister L, *Anal Chem.*, 1956, **28**, 1484-1485.
33. Kola I and Landis J, *Nat Rev Drug Dis.*, 2004, **3**, 711-715.